

Effect of Telomere and Telomerase Interactive Agents on Human Tumor and Normal Cell Lines¹

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ABSTRACT

Shortening of telomeres along with an up-regulation of telomerase is implicated in the immortality of tumor cells. Targeting either telomeres or telomerase with specific compounds has been proposed as an anticancer strategy. Because telomerase activity and telomeres are found in normal cells, telomere or telomerase targeting agents could induce side effects in normal tissues. We evaluated the effects of telomere and telomerase interactive agents in human tumor and normal cell lines to try to determine the potential side effects those agents might induce in patients. Toxicity of the G-quadruplex interactive porphyrins (TMPyP4, TMPyP2) and azidothymidine (AZT) were tested using a cell-counting technique against normal human cell lines (CRL-2115 and CRL-2120, fibroblasts; NHEK-Ad, adult keratinocytes; CCL-241, small intestinal cells; NCM 460, colonic mucosal epithelial cells) and human tumor cell lines (MDA-MB 231 and Hs 578T, breast cancer; SK-N-FI, neuroblastoma; HeLa, cervix cancer; MIA PaCa-2, pancreatic cancer; HT-29 and HCT-116, colon cancer; DU 145, prostatic cancer cell line). Telomerase activity of these cell lines was measured by a non-PCR-based conventional assay. The effects of TMPyP2, TMPyP4, and AZT were also evaluated against normal human bone marrow specimens, using a granulocyte-macrophage colony-forming assay (CFU-GM).

AZT showed very low cytotoxic effects against normal and tumor cell lines, with the IC₅₀ values above 200 μ M. The IC₅₀ values for TMPyP2 and TMPyP4 in normal human cell lines were in the range of 2.9–48.3 μ M and 1.7–15.5 μ M, respectively, whereas in tumor cell lines the IC₅₀ values were 11.4–53 μ M and 9.0–28.2 μ M, respectively. Within the tissue types, keratinocytes were more sensitive to TMPyP4 than fibroblasts, and small intestinal cells were more sensitive than colonic mucosal epithelial cells. The IC₅₀ for TMPyP2 and TMPyP4 in the normal marrow colony-forming assays were 19.3 ± 5.1 μ M and 47.9 ± 1.0 μ M, respectively. In conclusion, the *in vitro* cytotoxicity of the telomere interactive agent TMPyP4 is comparable in human tumor and normal cell lines, which indicates that TMPyP4 could have effects on normal tissues.

INTRODUCTION

Cellular senescence, defined by the limited proliferative capacity of normal cells, enables maintenance of homeostasis of the human body. Although some cells become transformed through various processes (1), transformed cells eventually undergo a proliferative crisis followed by cell death. Tumor cells must overcome this regulatory mechanism and immortalize to proliferate indefinitely. One of the most important processes of immortalization is the reactivation of telomerase activity to maintain the telomere length (2–4).

Shortening of telomeres along with an up-regulation in telomerase is implicated in the immortality of tumor cells (3–5). Targeting either telomeres or telomerase with specific compounds has been proposed as an anticancer strategy with tumor specificity (6–9). Recent understanding of the spatial structure of telomeric DNA, folded into G-quartet structures, led to the design of compounds interacting more specifically with the telomeric G-quartet, which may influence the extent of telomere elongation and finally induce the death of tumor cells (9, 10). On the basis of this information, the porphyrin TMPyP4 was developed as a candidate telomere interactive agent that interacts with the G-quartet specifically by an external stacking interaction. Meanwhile, the porphyrin TMPyP2, an isomer of TMPyP4, has demonstrated a different interaction with the G-quadruplex from TMPyP4 because it is sterically hindered from an external stacking (11). In addition, TMPyP4 has a telomerase-inhibitory effect in a cell-free system and in intact cancer cells (10, 12). TMPyP4 is a promising agent targeting both telomere and telomerase.

Telomerase is composed of a template RNA and catalytic subunit, hTERT, which has been shown to be closely related to other reverse transcriptases (13, 14). To achieve more specific targeting of telomerase, there have been many *in vitro* and *in vivo* trials using peptide nucleic acids, oligonucleotides as an

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antisense, or using AZT³ as an inhibitor of reverse transcriptase (15–17). In our previous reports, we showed that these telomere and telomerase interactive agents affected the proliferation rates and induced chromosomal instability not only in *in vitro* cell lines but also in a sea urchin embryo *in vivo* system, which is an effective model to evaluate the biological effects of novel agents (18).

To date, telomerase activity has been detected in some normal cells, including peripheral blood, cord blood and bone marrow lymphocytes, the basal layer of the skin, crypt cells of the proliferating intestinal epithelium, endometrium, proliferating endothelium, and in some benign breast epithelium (19–27). Although the telomerase activity in those tissues is lower than in most tumors and because all cells have telomeres, the telomere and telomerase targeting agents could induce side effects in these normal tissues. Conceivably, a combination of chemotherapeutic agents with these telomere and telomerase interactive agents could exaggerate the side effects in proliferating normal tissues. For the present study, we have evaluated the effects of telomere and telomerase interactive agents against human tumor and normal cell lines to try to predict the side effects those agents may induce in patients.

MATERIALS AND METHODS

Cell Lines. All of the human tumor cell lines (MDA-MB 231 and Hs 578T, breast cancer; SK-N-FI, neuroblastoma; HeLa, cervix cancer; MIA PaCa-2, pancreatic cancer; HT-29 and HCT-116, colon cancer; and DU 145, prostatic cancer cell line), two normal human skin fibroblasts (CRL-2115 and CRL-2120) and one normal intestinal cell line (CCL-241) were obtained from American Type Culture Collection (Rockville, MD). An adult normal human keratinocyte cell line (NHEK-Ad) was obtained from Clonetics Corporation (San Diego, CA). The normal human colonic epithelium cell line (NCM 460; Ref. 28) was purchased from INCELL Corporation (San Antonio, TX). All of the cell lines were grown according to the supplier's instructions. The characteristics of the cell lines that were used for the study are summarized in Table 1.

Telomere and Telomerase Interactive Agents. The G-quadruplex interactive tetracationic porphyrin (TMPyP4) and the positional isomer, TMPyP2 (used as a control of interest), were obtained from Midcentury (Posen, IL). All of the experiments with porphyrins were performed under minimum exposure to light except for the photoactivation experiment. The reverse transcriptase inhibitor, AZT, was purchased from Sigma Chemical Co (St. Louis, MO).

Determination of Telomerase Activity. Telomerase activity of normal human cell lines was measured using a non-PCR-based, conventional telomerase assay with 5'-biotinylated d(TTAGGG) as a telomere primer (29). Briefly, cell extracts were obtained from 1×10^7 cell pellets after washing with PBS. The cell pellet was washed once with ice-cold washing buffer [10 mM HEPES-KOH (pH 7.5), 1.5 mM MgCl₂, 10 mM KCl, and 1 mM DTT] and then pelleted again at $10,000 \times g$ for 1 min at

Table 1 Characteristics of the cell lines

Name	Origin	Source	Passages
Normal human cell lines			
CCL-241	Intestinal epithelium	ATCC	27–30
NCM-460	Colonic epithelium	INCELL Co.	38–40
CRL-2115	Skin fibroblast	ATCC	7–9
CRL-2120	Skin fibroblast	ATCC	7–9
NHEK-Ad	Adult keratinocyte	Clonetics Co.	3–4
Human tumor cell lines			
MDA-MB-231	Breast cancer	ATCC	
HS578T	Breast cancer	ATCC	
SK-N-FI	Neuroblastoma	ATCC	
HeLa	Cervix cancer	ATCC	
MIA-Paca-2	Pancreatic cancer	ATCC	
HT-29	Colon cancer	ATCC	
HCT-116	Colon cancer	ATCC	
DU 145	Prostatic cancer	ATCC	

4°C. The pellet was resuspended in 100–400 μ l of ice-cold lysis buffer containing 10 mM Tris-HCl (pH 7.5), 1 mM MgCl₂, 1 mM EGTA, 0.1 mM phenyl-methylsulfonyl fluoride, 5 mM β -mercaptoethanol, 1 mM DTT, 0.5% 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate, 10% glycerol and 40 UI/ml RNase guard. After incubation on ice for 30 min, the lysates were transferred to polyallomer tubes (Beckman, Fullerton, CA) and spun at $100,000 \times g$ for 1 h at 4°C in a tabletop ultracentrifuge. The supernatants were stored at –80°C in 10% glycerol. Protein concentrations were determined by the Bradford assay (Bio-Rad, Hercules, CA). All of the cell line extracts were normalized to the same protein concentration of 1 mg/ml.

For the telomerase reaction, the reaction mixture (20 μ l) containing 4 μ l of cell lysate, 50 mM Tris acetate (pH 8.5), 50 mM potassium acetate, 1 mM MgCl₂, 5 mM β -mercaptoethanol, 1 mM spermidine, 1 μ M telomere primer, 1.5 M [α -³²P]dGTP (800 Ci/mmol), 2 mM dATP, and 2 mM dTTP were incubated at 37°C for 1 h. The reactions were terminated by adding 20 μ l of streptavidin-coated Dynabead suspension containing 10 mM Tris-HCl (pH 7.5) and 2 M NaCl. The beads complexed selectively with the 5'-biotinylated DNA. The complex was separated from the suspension using a magnet (Dynal MPC) and washed several times with washing buffer (1 M NaCl) to eliminate [α -³²P]dGTP background. Telomerase reaction products were separated from the magnetic beads by protein denaturation with 5.0 M guanidine hydrochloride at 90°C for 20 min. After ethanol precipitation, the reaction products were analyzed by 8% PAGE. Telomerase activity in HeLa cell extracts were used for reference and defined as 100% activity.

Cytotoxicity Assay. A cell proliferation assay was performed by a cell-counting technique using Coulter ZM counter (Coulter Electronics, Luton, United Kingdom). Exponentially growing 5×10^3 cells in 1 ml of medium were plated in 24-well microtiter plates on day 0. On day 1, 1 ml of fresh medium containing various concentrations of the drugs was added to the plate. On days 4 and 7, the cells were trypsinized, and the cells were counted with the Coulter counter. Each assay was performed in duplicate. The IC₅₀ for the drug was determined by the EZ50 software program (Perrella Scientific Inc., Amherst, NH).

³ The abbreviations used are: AZT, azidothymidine; CFU-GM, granulocyte-macrophage colony-forming unit(s).

Photoactivation Effects on the Cytotoxicity of Porphyrins. Because porphyrins are known photosensitizers and can cause nonspecific DNA damage on light exposure, we evaluated the effects of photoactivation of TMPyP2 and TMPyP4 on normal cells. The cells were cultured in 24-well plates in the continuous presence of the various concentrations of compounds under limited light exposure ("dark"), which is a standard way of handling the porphyrins in all of the bioassays in our laboratories. Photoactivation of cells treated with porphyrins in separate plates was achieved by a daily 15-min exposure for 5 days to a 60-W light source 12 inches above the flasks ("light"). This light treatment was not associated with any thermal effects on the cells. The IC₅₀ values were determined by cell counts in both sets of plates.

CFU-GM Assay. The cytotoxic effects of telomere and telomerase interactive agents on normal human bone marrow colony-forming units were assessed by examining the number of CFU-GM-derived colonies. Briefly, bone marrow mononuclear cells were collected from normal healthy bone marrow donors after informed consent as described previously (30). These studies were approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. Nonadherent bone marrow cells were recovered after 2-h incubation in plastic tissue culture dishes and plated in 35-mm tissue culture dishes at 10⁵ cells/ml in a 1-ml volume of 1.5% methylcellulose (Aldrich Chemical Co., Milwaukee, MI) supplemented with 20% heat-inactivated FCS (Tissue Culture Biologicals, Tulare, CA), BSA (Sigma Chemical Co.), 1.0 ng/ml recombinant human granulocyte macrophage colony-stimulating factor and different concentrations of the drugs. Each assay was performed in triplicate. The number of the colonies was counted after 14 days incubation in 5% CO₂ at 37°C.

RESULTS

Selection of the Telomere and Telomerase Interactive Agents and Cell Lines. Recent understanding of the spatial structure of telomeric DNA, folded into G-quartet structures, led to the design of compounds interacting more specifically with telomeric G quartets, which may influence the extent of telomere elongation and finally induce the death of tumor cells (9–11). Among many candidate compounds, the cationic porphyrin, TMPyP4 [5,10,15,20-tetra(*N*-methyl-4-pyridyl) porphinchloride], was found to effectively stack with the G tetrads to stabilize quadruplex DNA. In addition, TMPyP4 has the significant telomerase inhibitory effects not only in a HeLa cell-free system but also in intact breast cancer cells in a dose- and time-dependent manner (12). In the present study, we have chosen TMPyP4 as a specific G-quadruplex interactive agent. As a control agent of interest, we used TMPyP2, the isomer of TMPyP4 that has a less specific interaction with G-quadruplexes. Recent reports provide evidence that the catalytic subunit of telomerase (hTERT) is more important for the capacity of telomerase to confer immortalization on tumor cells than the RNA subunit of telomerase (13, 14). The hTERT subunit of telomerase is very similar to reverse transcriptase; therefore, AZT has been selected as a telomerase-specific inhibitor that works through inhibition of reverse transcriptase.

The porphyrins are already used clinically in dermatology

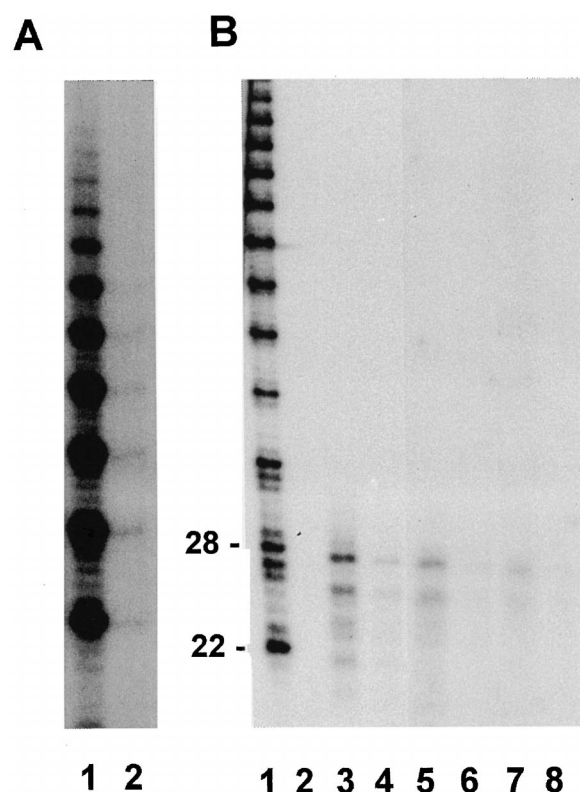


Fig. 1 Telomerase activity of normal human cell lines as assayed by a non-PCR-based conventional assay. **A**, Lane 1, HeLa cell extract; Lane 2, NCM 460 (colonic mucosal epithelium) cells. **B**, Lane 1, HeLa cell extract; Lane 2, HeLa extract with RNase treatment; Lane 3, CRL-2115 (skin fibroblast) cell line; Lane 4, CRL-2115 with RNase treatment; Lane 5, CRL-2120 (skin fibroblast) cell line; Lane 6, CRL-2120 with RNase treatment; Lane 7, Ad-NHEK (adult keratinocyte) cell line; Lane 8, Ad-NHEK with RNase treatment.

and oncology (31), and some interaction between porphyrins and normal skin cells might be expected. Telomerase activity is detected not only in the basal cell of the skin but also in the keratinocyte (21). Therefore, we selected normal human skin fibroblasts and adult human keratinocytes as target cell lines. Intestinal epithelium is a rapidly proliferating tissue that is often susceptible to chemotherapy and is known to have telomerase activity. We also selected a normal human intestinal cell line and a normal colonic mucosal epithelial cell line for prediction of possible side effects on those rapidly proliferating cells. To evaluate the effect of telomere and telomerase interactive agents on hematopoietic cells more precisely, we studied normal human bone marrow cells using a colony forming assay. As positive controls, we used eight human tumor cell lines with known various telomerase activities and telomere lengths (Table 1).

Determination of Telomerase Activity. All of the tumor cell lines that were used for this study showed telomerase activity. Telomerase activity levels for the normal human cell lines, found by using the non-PCR based conventional telomerase assay are shown in Fig. 1. All of the normal human cell lines showed relatively weak telomerase activity compared with

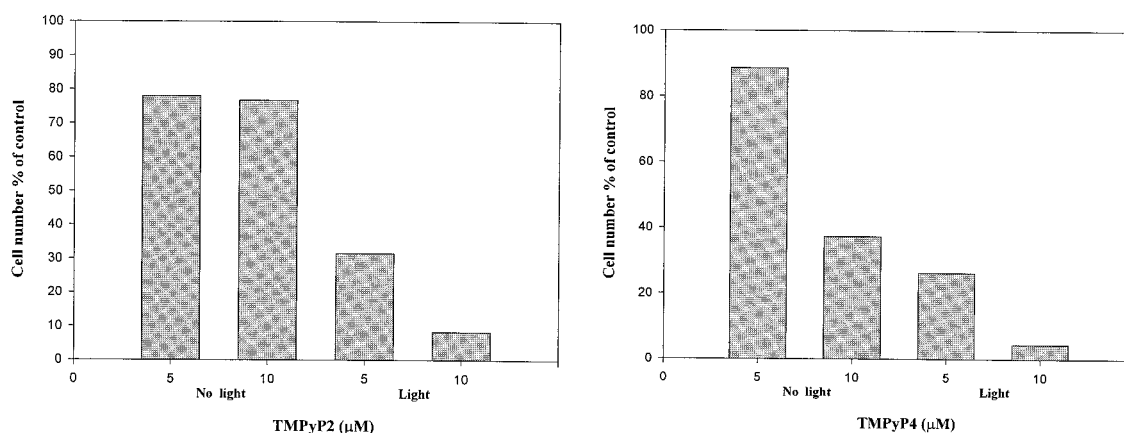


Fig. 2 The light effects in growth inhibition of the porphyrins in normal fibroblast cell line (CRL-2115). The cell numbers were counted by Coulter counter after the cells were grown in 24-well plates with the various concentrations of the TMPyP2 and TMPyP4 after incubation under the light for 15 min 5 days a week.

Table 2 Mean IC_{50} for inhibition of cell growth by telomere and telomerase interactive agents on tumor cell lines using cell-counting technique

Cell line	Tumor type	Relative telomerase activity ^a	IC_{50} (μM)		
			TMPyP2	TMPyP4	AZT
MDA-MB-231	Breast cancer	+++	53.0	25.0	>200
HS ₅₇₈ T	Breast cancer	+	11.4	9.0	>200
SK-N-FI	Neuroblastoma	++	16.3	42.0	>200
HeLa	Cervix cancer	++	22.8	18.5	>200
MIA-Paca-2	Pancreatic cancer	++	32.4	21.9	>200
HT-29	Colon cancer	+	31.8	11.0	>200
HCT-116	Colon cancer	+	37.4	28.2	>200
DU 145	Prostatic cancer	±	26.5	21.6	>200

^a Relative telomerase activity compared with the activity of HeLa cell extract level of ++.

the activity of HeLa cell extracts used as a reference. Among them, only one colonic mucosal epithelial cell line showed weak processive telomerase activity in this conventional assay. The other four cell lines showed nonprocessive telomerase activity. Telomerase activity in skin fibroblast (CRL-2115) cells was relatively strong (Fig. 1).

Photoactivation Effects on the Cytotoxicity of Porphyrins. We tested the photoactivation effects on two different normal fibroblast cells. The IC_{50} values for TMPyP2 and TMPyP4 under the dark condition described above were 28.1 μM and 7.3 μM in CRL-2115 cells and 22.6 μM and 15.5 μM in CRL-2120 cells. With these dark conditions, TMPyP4 was more cytotoxic than TMPyP2. After the treatment with light for 5 days as described above, the IC_{50} values for TMPyP2 and TMPyP4 were decreased to 2.25 μM and 1.5 μM, respectively, in CRL-2115 cells and 11.8 μM and 11.6 μM, respectively in CRL-2120 cells. When we treated the CRL-2115 fibroblast cells with 5 μM and 10 μM of TMPyP2 and TMPyP4, TMPyP2 did not substantially affect the cell growth with limited light. However, when exposed to light, TMPyP2 induced the same cytotoxic effect as TMPyP4 did. Because the light induced the same cytotoxicity with TMPyP2 and TMPyP4, it suggested to us that there is a nonspecific effect of these agents related to the light (Fig. 2).

Cytotoxicity Assay. The cell proliferation assay results for the eight tumor cell lines and five normal cell lines were compared based on the cell-counting technique. Because of the light sensitivity of porphyrins, we handled the cell plates under the limited light conditions described above. The IC_{50} values for TMPyP2 and TMPyP4 in tumor cell lines were in the range of 11.4–53 μM and 9.0–28.2 μM, respectively, whereas AZT showed very low cytotoxic effects against tumor cell lines (Table 2). There was no apparent correlation between cytotoxicity and the tumor types or telomerase activities of the tumor cell lines. The IC_{50} values for TMPyP2 and TMPyP4 in normal human cell lines were in the range of 2.9–48.3 μM and 1.7–15.5 μM, respectively, whereas AZT showed very low cytotoxic effects just as in tumor cell lines (Table 3). Within the tissue types, keratinocytes were more sensitive to TMPyP4 than fibroblasts, and the small intestine-derived cells were more sensitive than colonic mucosal epithelial cells. TMPyP4 was more cytotoxic than TMPyP2 in tumor and normal cells.

CFU-GM Assay. The cytotoxic effects of TMPyP4 on hematopoietic cells from 10 healthy normal human bone marrow donors were assessed by examining the number of CFU-GM. The IC_{50} for TMPyP4 in the normal marrow colony-forming assays was 47.9 μM (Fig. 3A). The effects of TMPyP2 and AZT were evaluated with four normal human bone marrow

Table 3 Mean IC₅₀ for inhibition of cell growth by telomere and telomerase interactive agents on normal human cell lines using cell-counting technique

Cell line (tissue type)	IC ₅₀ (μM)		
	TMPyP2	TMPyP4	AZT
CRL-2115 (fibroblast) ^a	28.1	7.3	>200
CRL-2120 (fibroblast) ^a	22.6	15.5	>200
NHEK-Ad (keratinocyte) ^b	2.9	1.7	>200
CCL-241 (intestine) ^b	48.3	5.3	>200
NCM-460 (colon) ^b	11.1	7.5	>200

^a Mesenchymal cells.

^b Epithelial cells.

cells, and the IC₅₀ values were 19.3 μM and 86.9 μM, respectively (Fig. 3, B and C).

DISCUSSION

It is increasingly apparent that telomerase activity is present in many normal cells including hematopoietic cells, keratinocytes, crypt cells of intestinal epithelium, and others (19–27). Although the telomerase activity in those normal tissues is weaker than tumor tissues, and its relevance is yet to be clarified, telomerase-targeting agents could induce side effects in those normal tissues. Also, telomeres in normal cells may be affected by telomere interactive agents. In preparation for the introduction of a telomere and telomerase interactive agent into clinical trials, we evaluated the possible effects of such agents on human tumor and normal cell lines to try to predict the side effects those compounds may induce in cancer patients.

As baseline information, all of the five normal human cell lines showed weak telomerase activity. This is not surprising because, although these cell lines are known as normal in character with a limited life span, they are growing in culture under stable conditions. Interestingly, only one cell line (a colonic mucosal epithelial cell line) showed a weak processive telomerase activity. Possible explanations for this processive telomerase activity in colonic epithelial cells are: (a) this cell line has properties of stem cells of the colonic epithelium; and (b) this cell line has been selected for continuous *in vitro* culture (it was tested at 38th passage), which means that it could possibly be in the process of immortalization but maintains a colon cell phenotype.

Porphyrins are photosensitizing agents that are effectively used in diagnosis and therapy in dermatology and oncology (31). Keratinocytes and skin cells are the normal tissues expressing telomerase activity, and those cells may be more affected by porphyrins because of photosensitivity. On the basis of this assumption, we evaluated the photoactivation effects on the cytotoxicity of porphyrins on normal cell lines. Indeed, TMPyP2 and TMPyP4 showed more toxic effects under light exposure. After the light treatment, the cytotoxicity of TMPyP2 was similar to that of TMPyP4, which suggested that the non-specific cytotoxicity of these drugs could be related to the light. Therefore, one must pay attention to possible photosensitivity side effects from telomere and telomerase interactive porphyrin treatment, especially in the sun-exposed skin.

Using the cell-counting techniques, we observed similar

toxicity of the agents tested in normal cell lines compared with tumor cell lines. We also observed that TMPyP2 was less cytotoxic in normal and tumor cell lines in comparison with TMPyP4. The cytotoxic effect of telomere and telomerase interactive agents against normal human marrow cells in the colony-forming assay showed the same range of IC₅₀ as in other normal cell lines.

There was no difference in cytotoxicity according to the tumor types or telomerase activities of the tumor cell lines, consistent with our previous results (12). Because the main mechanism of action of TMPyP4 is via interaction with telomeric DNA, it is not surprising that there is not a correlation between the cytotoxic effect of TMPyP4 and the levels of telomerase activity. Furthermore, the lack of cytotoxicity with AZT, which inhibits telomerase activity, is not surprising, given that the direct inhibition of telomerase activity is not enough to induce cell growth inhibition in a short period of time. Comparable toxicity of porphyrins in tumor and normal cells supports the hypothesis that these compounds target telomeres rather than telomerase.

Because all of the cells have telomeres, tumor selectivity of these telomere and telomerase interactive agents is the main problem to solve. Possible ways to enhance tumor selectivity by these agents include: (a) tumor cells may have different types of the G-quadruplexes, which could be the specific target of TMPyP4; (b) because the maintenance of telomeres is known to be regulated by the telosome, the complex of telomere, telomerase, and the regulatory proteins of telomerase or telomeres [such as TRF1,2; hnRNP A1; or TP (telomerase binding protein); Refs. 32–34], there may be a unique role of telomere- and telomerase-related proteins to form G-quadruplexes with telomeric DNA in tumor cells, which may give some selectivity; (c) porphyrins are known to be rapidly and preferentially taken up by the tumor cells (35, 36); and (d) rapidly proliferating cells, such as some tumor cells, may give more opportunity for the porphyrins to interact with G quadruplexes formed by single-strand overhangs.

Up to now, telomere and telomerase interactive agents have been regarded as probable cytostatic agents that could be more effective in combination with common chemotherapeutic agents. Some limitations of chemotherapy are side effects on proliferating normal somatic cells, such as bone marrow and mucosal cells. The results from our study indicate that the spectrum of side effects of telomere and telomerase interactive agents and chemotherapeutic agents might overlap. Certainly, combining telomere and telomerase interactive agents with chemotherapeutic agents will have to be done with caution.

There are some difficulties inherent in using *in vitro* normal cell systems to evaluate the cytotoxicities of telomere and telomerase interactive agents: (a) normal cells are fragile in *in vitro* culture systems. Thus, the normal cell lines that we used could be more susceptible to the agents tested because of this fragility; (b) normal cells have a limited life span. Because of that finite life span, we could not evaluate the effect of long term culture. This study provides us only with a spectrum of short-term toxicity against normal cell lines; (c) normal cells are in the process of senescence. The cytotoxic effect of telomere and

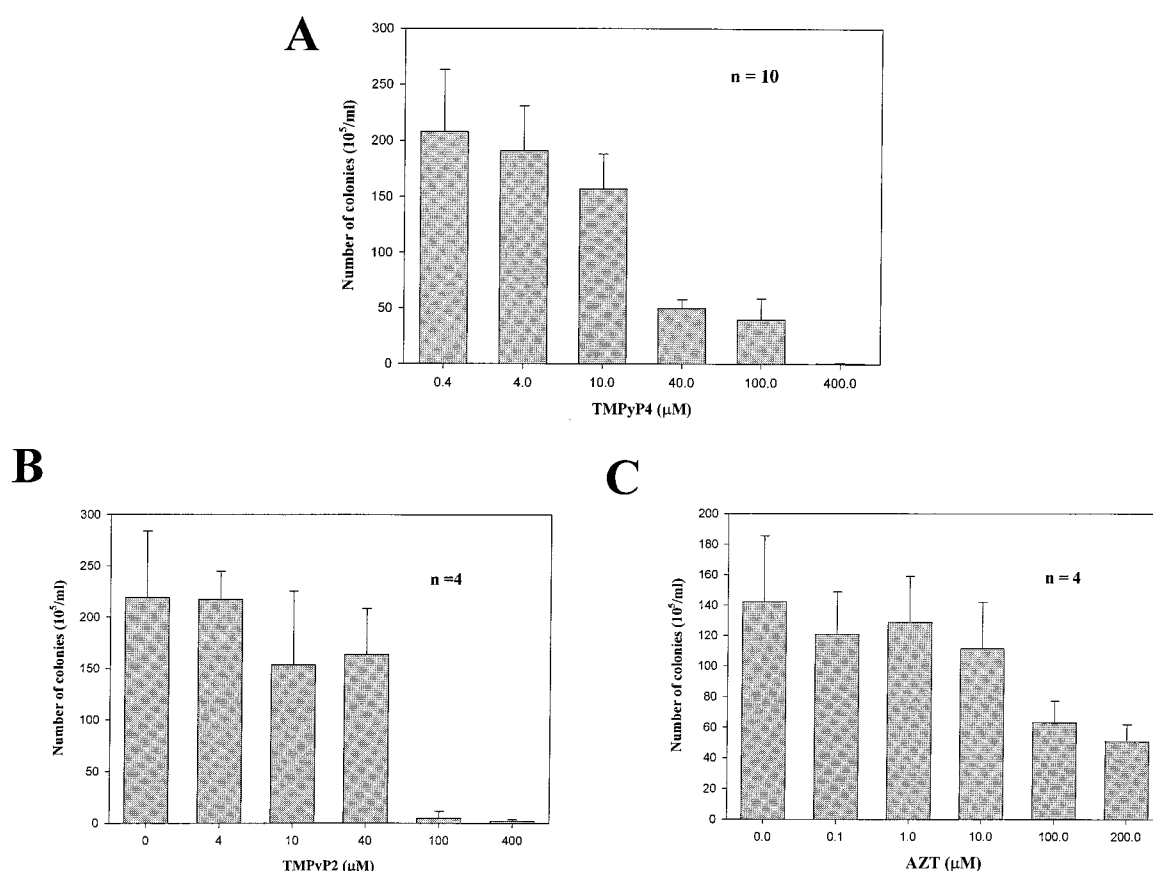


Fig. 3 The cytotoxic effects of TMPyP4 (A), TMPyP2 (B), and AZT (C) were measured on normal human bone marrow cells by using a CFU-GM assay. Each assay was performed in triplicate. The number of the colonies was counted after 7 days incubation in a 5% CO₂ incubator at 37°C.

telomerase interactive agents in the normal cell lines could be exaggerated in the process of senescence.

Despite the above obstacles, our data indicate that the porphyrin class of telomere and telomerase interactive agents may affect normal cells. To enhance the target specific effects, additional studies to exploit the mechanism of action of these agents are warranted. A substantial program is under way to develop nonphotosensitive porphyrin analogues to reduce the potent side effects and to enhance the tumor specificity of these agents.

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